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PB-251 931

A QUANTITATIVE METHOD FOR TOXAPHENE BY GC-CI-MS SPECIFIC ION MONITORING

Environmental Research Laboratory

March 1976

120221

PB 251 931

EPA-600/4-76-010
March 1976

Environmental Monitoring Series

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Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Athens, Georgia 30601

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/4-76-010	2.
3. RECIPIENT'S ACCESSION NO.	
4. TITLE AND SUBTITLE A Quantitative Method for Toxaphene by GC-CI-MS Specific Ion Monitoring	5. REPORT DATE March 1976 (Issuing Date)
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Alfred D. Thruston, Jr.	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Athens, Georgia 30601	10. PROGRAM ELEMENT NO. 1BD612
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Same as above	13. TYPE OF REPORT AND PERIOD COVERED In-house
	14. SPONSORING AGENCY CODE EPA-ORD
15. SUPPLEMENTARY NOTES	

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS PRICES SUBJECT TO CHANGE

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Pesticides, Mass Spectrometry,	*Toxaphene, *Pesticide analysis, Analytical technique, Chlorinated pesticides	05A

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 30
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE $4.00-2.25

EPA Form 2220-1 (9-73)

☆ U.S. GOVERNMENT PRINTING OFFICE: 1976-657-695/5383 Region No. 5-11

EPA-600/4-76-010
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A method was developed for the identification and quantification of toxaphene using a Specific Ion Monitoring (SIM) program with GC-CI-MS. Interferences from DDT's and Arochlor 1260 are eliminated or minimized. GC-CI-MS was also used to distinguish toxaphene from strobane.

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SECTION I

INTRODUCTION

Toxaphene is one of the most widely used chlorinated hydrocarbon insecticides in the United States.[1] Although it is very effective in controlling insects in agriculture, it also creates hazards in the environment. Several studies are reported in the literature concerning the effects of the pesticide on aquatic life. Recently, ng-per-liter levels of toxaphene were shown to cause a spine defect in fish.[2] Accurate quantitative methods for toxaphene analysis are therefore needed to determine its persistence in the aquatic environment.

Toxaphene is manufactured by chlorinating camphene, which results in a complex mixture of approximately 177 chlorinated isomers.[3] The overall average elemental composition is $C_{10}H_{10}Cl_8$ with the major components being hepta-, octa-, and nonachlorobornanes. Gas chromatography (GC) has been regarded as the most useful technique for toxaphene residue analysis. Analysis for this complex mixture, however, is often subject to interferences from the pesticides DDE, TDE, or DDT or from the very common polychlorinated biphenyls (PCB's, trade name Arochlors), which often make necessary extensive clean-up of the extract before GC analysis.[4,5]

Gas chromatography-mass spectrometry (GC-MS), is a valuable technique in pesticide analysis. Although in the electron impact (EI) mode, GC-MS is generally insensitive to trace amounts of toxaphene, studies by Holmstead, et al.[3] and Stallings and Huckins[6] show that the chemical ionization (CI) mode with GC-MS is particularly applicable to the analysis of toxaphene, especially using Limited Mass Reconstructed Gas Chromatograms (LMRGC) for identification.

The Specific Ion Monitoring (SIM) program, a limited mass data acquisition program developed at Battelle Columbus Laboratories for EPA,[7] also increases the sensitivity of GC-MS detection. It therefore is an additional useful technique to be used along with the standard System 150 LMRGC. The program, evaluated at the Environmental Research Laboratory (formerly Southeast Environmental Research Laboratory) was found to be applicable to pesticide residue analysis.[8]

A study was therefore undertaken 1) to develop a sensitive quantitative GC-MS procedure (in the CI mode) using the SIM

program for determination of toxaphene residues in environmental samples, 2) to eliminate or minimize interferences common in GC toxaphene analysis, such as the DDT family or PCB's, and 3) to provide a way of differentiating toxaphene from the similar pesticide strobane. In parallel with this study, the Methods Development and Quality Assurance Research Laboratory in Cincinnati agreed to investigate the EI mode of GC-MS for toxaphene detection using LMRGC.

SECTION II

CONCLUSIONS

This method using the Specific Ion Monitoring (SIM) program with GC-CI-MS is rapid and accurate for the identification and quantification of toxaphene. Interferences from DDT's and Arochlor 1260 are eliminated or minimized. Toxaphene can be distinguished from strobane by utilizing GC-CI-MS techniques.

SECTION III

MATERIALS AND METHODS

INSTRUMENTATION

Mass spectral data were obtained with a Finnigan 9500 gas chromatograph and 1015D chemical ionization quadrupole mass spectrometer (GC-CI-MS) equipped with a continuous dynode multiplier and operated with the following conditions: 70 eV electron energy, 10^{-7} sensitivity range, 500 mA ionizing current, and a 2000 V electron multiplier. The chromatograph column was a 60 cm x 2 mm (I.D.) glass column packed with 3% SP2100 on 80/100 Supelcon AW. The methane carrier gas, which also served as the reagent gas, was adjusted to give a pressure of 1.0 torr in the ion source (about 20 ml/min flow through the column). The column was programmed from 160° to 250°C at 10°/min for each run.

SAMPLE PREPARATION

A stock solution of pesticide reference grade toxaphene (1 µg/µl) and one of the internal standard 2,4,2',5' tetrachlorobiphenyl (TCB) (1 µg/µl) were prepared. From these stock solutions, five mixtures were prepared, representing three ratios of toxaphene to TCB.

Solution	Solution Concentration (ng/µl)		Toxaphene:TCB Ratios
	Toxaphene	TCB	
1	30	0.6	50:1
2	20	0.6	33:1
3	10	0.6	17:1
4	7.5	0.15	50:1
5	2.5	0.15	17:1

A standard solution (1 µg/µl) of pesticide reference grade strobane in isooctane was also prepared.

DATA ACQUISITION ON SIM PROGRAM

Data acquisition for the SIM program is performed as described by Alford.[8]

Data acquisition parameters are specified by teletype communications between analyst and computer. Two m/e values for the internal standard TCB are entered as one set to be monitored, and integration times in milliseconds for that set are specified. Another set of m/e values and an integration time for toxaphene are entered as a second set to be monitored (initiated by typing "S2").

The "NO. POINTS" prompt specifies the number of times each set of masses is to be monitored before the computer adds the acquired data and stores the sum.

For a more detailed explanation of the procedure, see Appendix.

SECTION IV

EXPERIMENTAL AND DISCUSSION

The SIM program and standard System 150 were used to obtain data for the analysis for toxaphene, showing the effects of various interfering compounds and backgrounds. The 60-cm GC column was chosen to condense the range of toxaphene retention times for ease in quantification, while retaining the characteristic "toxaphene pattern" familiar to pesticide gas chromatographers. The analysis takes 9 minutes.

Tetrachlorobiphenyl was chosen as the internal standard since it elutes just before toxaphene and its CI spectrum (Figure 1) contains none of the masses of the early eluting toxaphene peaks. To keep the chart speed the same throughout the run, two masses were selected to be monitored. One of these masses should provide the strongest signal in the spectrum since the entire run is normalized to the strongest signal. For TCB, masses 291, 293, 295, or 321 are the possibilities. The TCB concentration may then be adjusted so that a chosen peak is the most intense peak in the spectrum.

Several compounds that eluted after toxaphene, including mirex and decachlorobiphenyl, were also considered as internal standards; however reproducable intensities for these compounds could not be obtained.

The CI-MS of toxaphene is characterized by the major fragments $[M-Cl]^+$, $[M-Cl-HCl]^+$, and $[M-Cl-2HCl]^+$. These ion clusters, which reflect the substitution patterns of the toxaphene chlorine isomers, are strongest centering around m/e 235, 271, 307, 343, 377, and 413. Figure 2 shows a reconstructed gas chromatogram (RGC) of toxaphene and two typical mass spectra. Masses 307 and 343 were chosen to be monitored by the SIM program because of their intensities and the absence of background interferences at these masses. These two masses also retain the familiar "toxaphene pattern" better than the 377 m/e, which also satisfies the interference and sensitivity criteria.

After GC conditions and the ions to be monitored were chosen, SIM program parameters were optimized. The most quantitative data were obtained when 2 internal standard masses and 2 toxaphene masses were monitored for 200 msec integration time at each mass. Three points were added before the sum of intensities was stored.

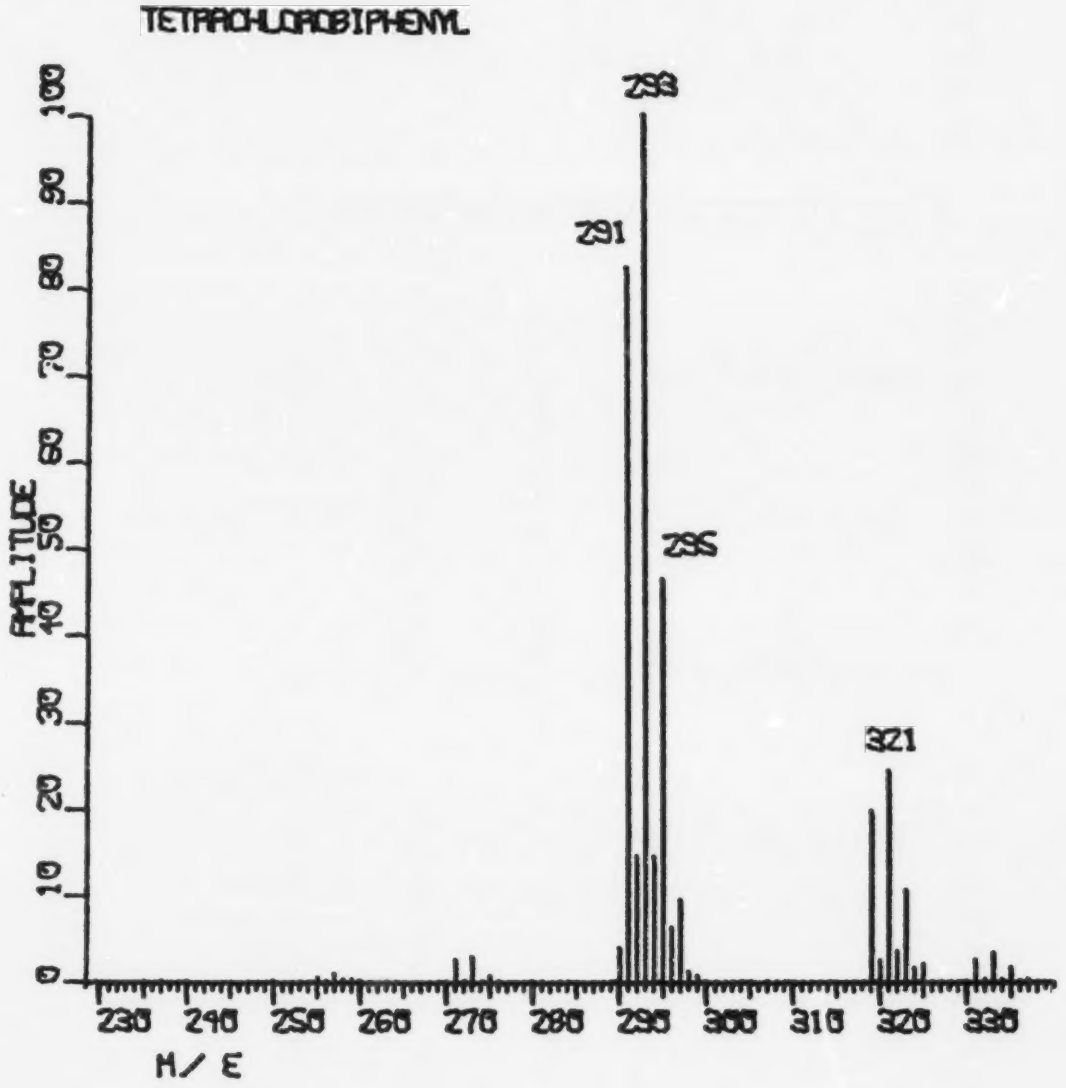


Figure 1. CI mass spectrum of 2,4,2',5'-tetrachlorobiphenyl

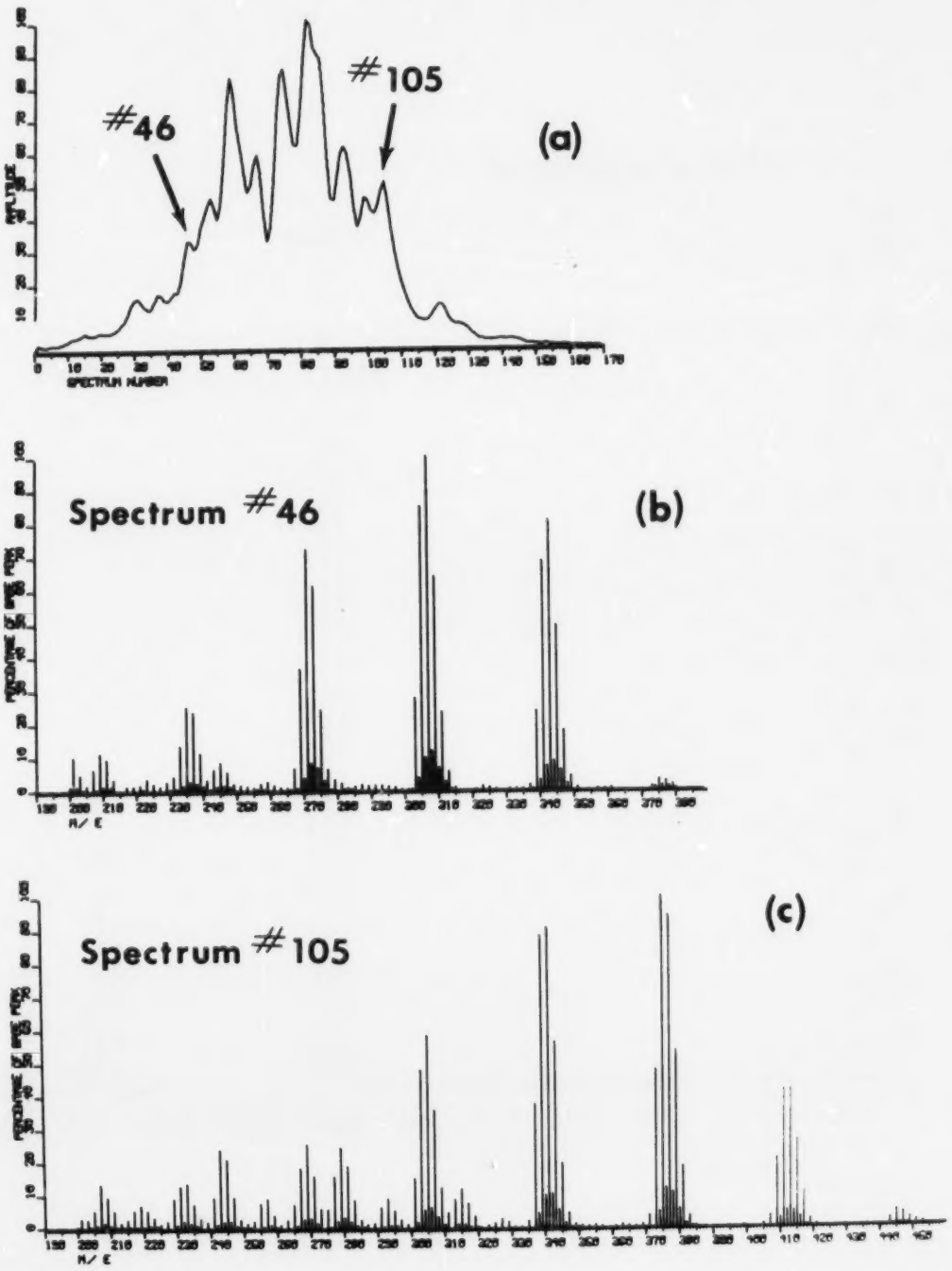


Figure 2. (a) Toxaphene RGC
(b) Spectrum of $C_{10}H_xCL_6$ Type
(c) Spectrum of $C_{10}H_xCl_9$ Type

The average of the peak heights of the two highest toxaphene peaks of each mass run gave the most reproducable quantitative results. Both the m/e 307 and m/e 343 curves gave similar intensities and either can be used for quantification. In practice, the m/e with least background interference should be used.

Two sensitivity ranges were observed for toxaphene analysis corresponding to a normal and a freshly cleaned mass spectrometer ion source. Figure 3 shows a standard curve for toxaphene using 6 consecutive injections (2 µl) of 30 ng/µl solution, 5 of 20 ng/µl, and 4 of 10 ng/µl. All instrumental conditions were kept as constant and optimum as possible.

After this series was done, the mass spectrometer ion source was taken apart and cleaned (a heavy load of samples caused a gradual decrease in sensitivity). After cleaning, the sensitivity of the mass spectrometer increased by about a factor of 4. Figure 4(a) shows the SIM program output (after cleaning) for 15 ng toxaphene and Figure 4(b) shows the output for 5 ng toxaphene. Figure 5 shows a standard curve for toxaphene at this new sensitivity using 6 consecutive injections (2 µl) of 7.5 ng/µl solution and 4 of 2.5 ng/µl.

NEW ORLEANS DRINKING WATER SAMPLE

The New Orleans Drinking Water Survey was undertaken in July 1974 to determine the organic compounds present in the finished water of the Carrollton Water Plant (city of New Orleans).[9] Eighty organic compounds were identified and quantitated in the 0.05 to 10 µg/l range. The carbon-chloroform extract from this survey was chosen as an ideal environmental sample to spike with toxaphene for testing the applicability of the GC-CI-MS technique for toxaphene determination.

Figure 6(a) shows the output for a 2 µl injection of the New Orleans Carrollton Plant Water carbon-chloroform extract concentrate (1 µl extract ≅ 25 ml water). Figure 6(b) shows the output for 2 µl of the New Orleans extract spiked with 5 ng toxaphene (equivalent to 0.1 µg/l in the original water). Figure 6(c) is the output for 2 µl of the New Orleans extract spiked with 15 ng toxaphene (equivalent to 0.3 µg/l). At the 5 ng toxaphene level the 307 m/e scan is obscured by background; however, the 343 m/e scan is quite recognizable as toxaphene (compare with Figure 4(b)). At the

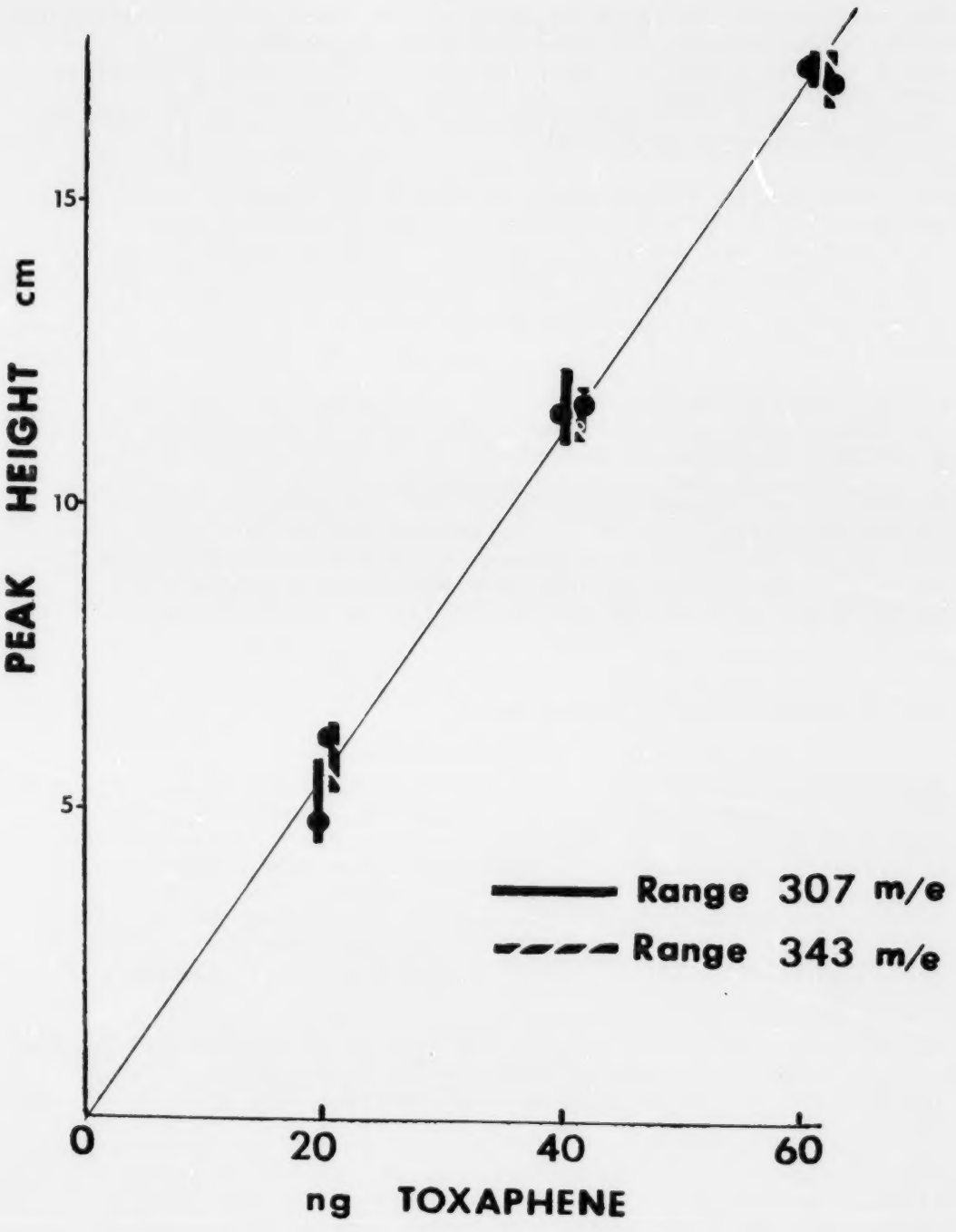


Figure 3. Standard curve of toxaphene before cleaning mass spectrometer ion source

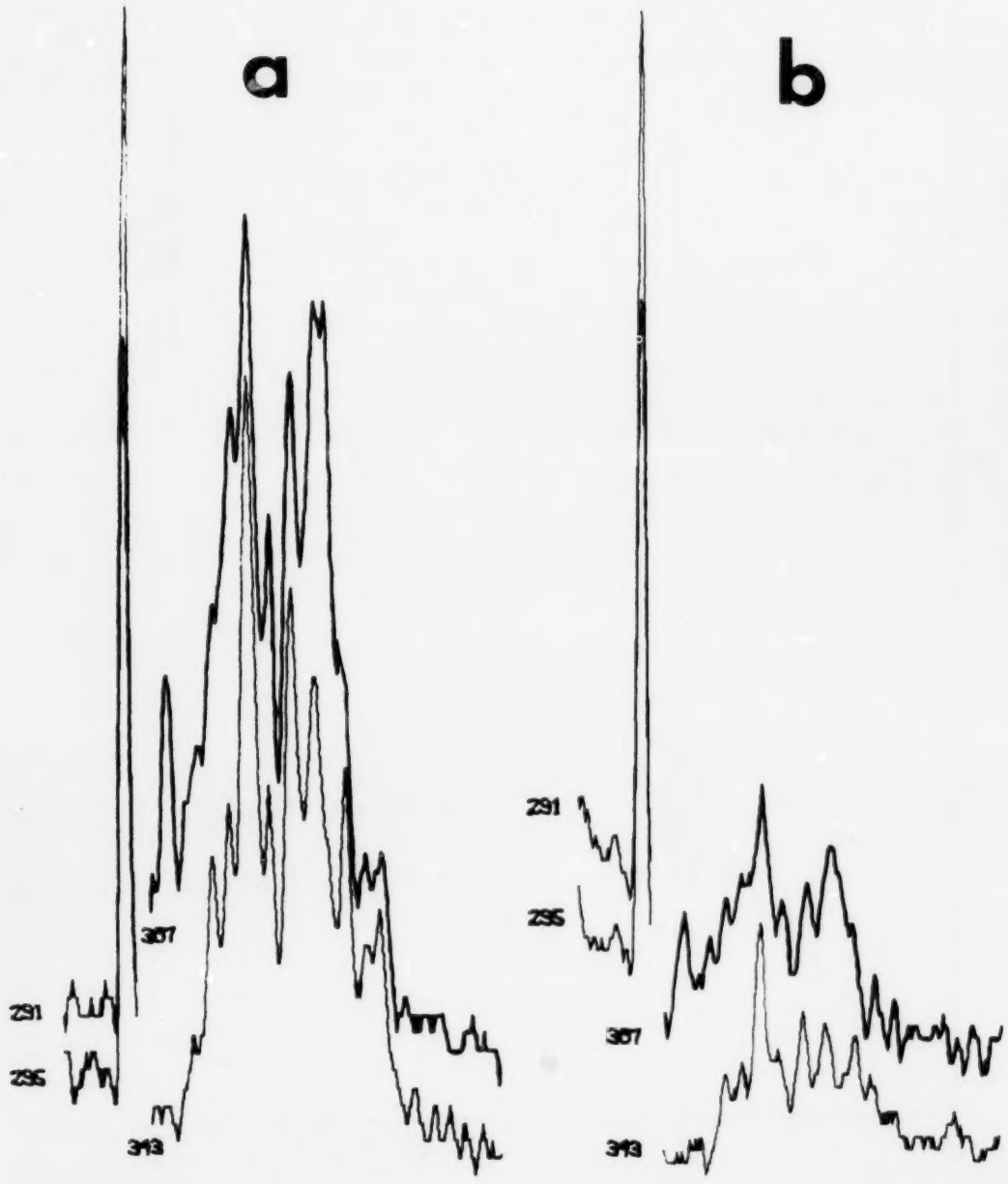


Figure 4. (a) SIM program output, 0.3 ng internal std. TCB, 15 ng toxaphene
(b) SIM program output, 0.3 ng internal std. TCB, 5 ng toxaphene

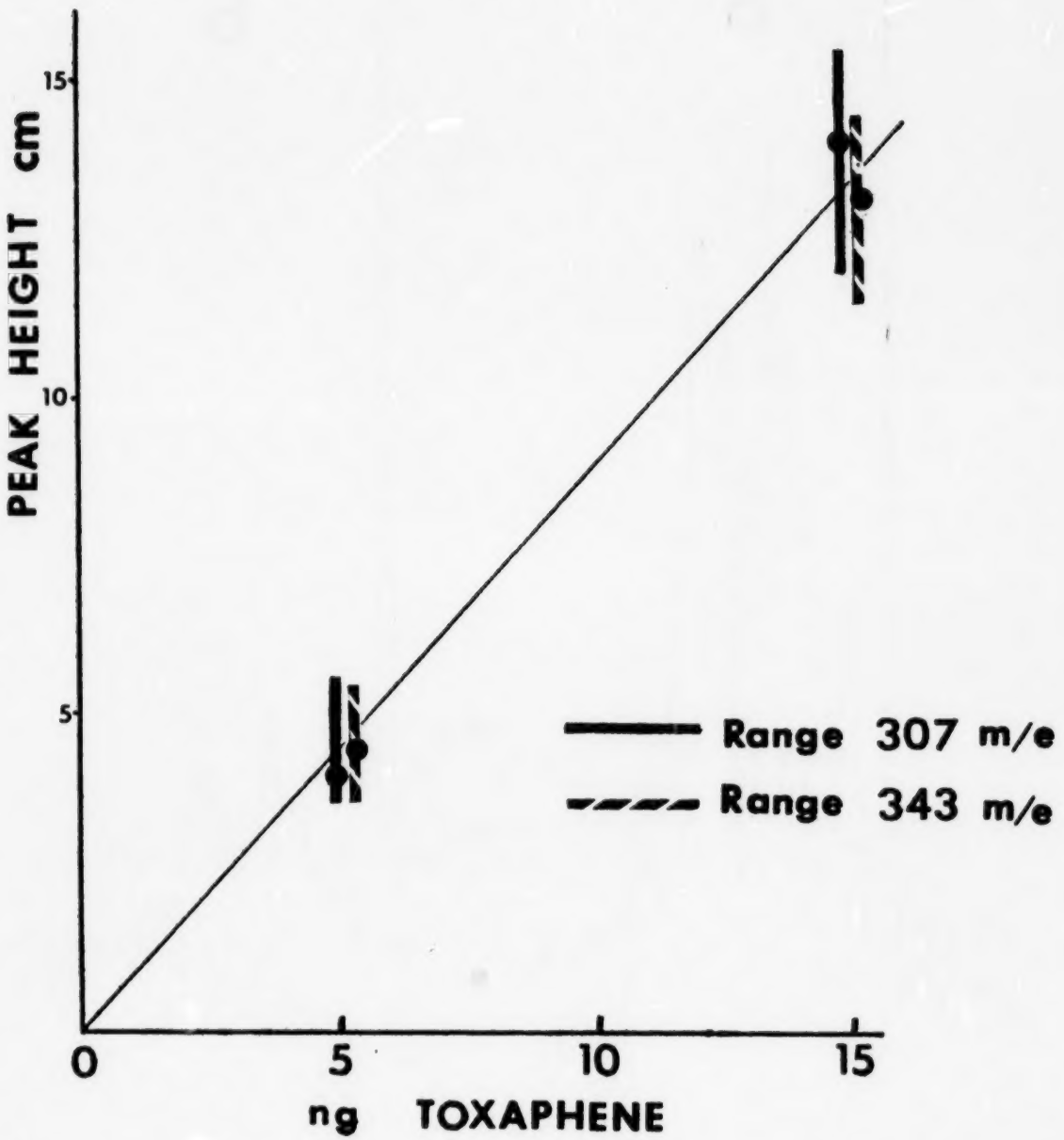


Figure 5. Standard curve of toxaphene after cleaning mass spectrometer ion source

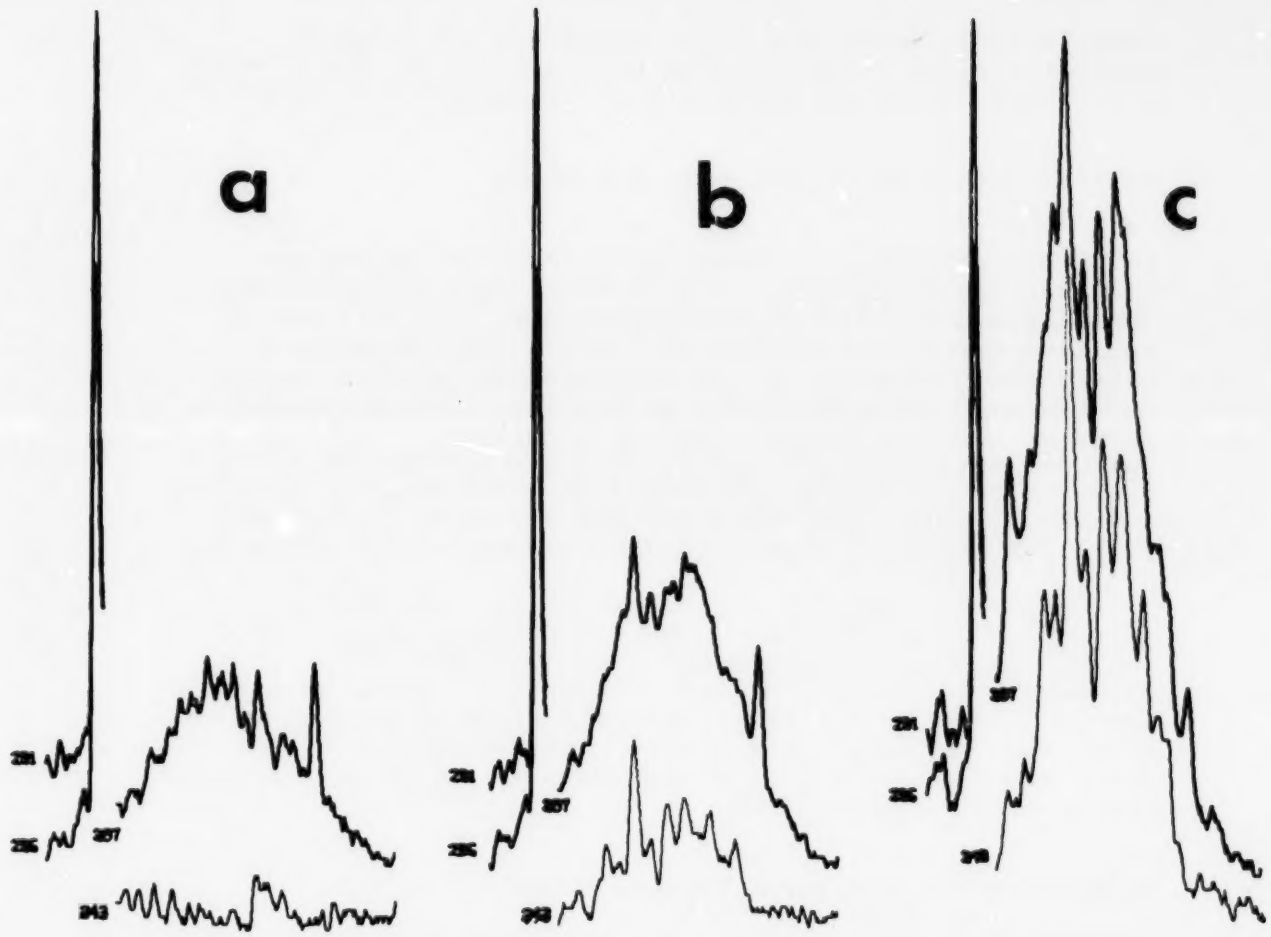


Figure 6. (a) New Orleans Drinking Water Extract. 2 µl of 1:25,000 $CHCL_3$ solution plus 0.3 ng internal standard TCB
(b) New Orleans Drinking Water Extract. 2 µl of 1:25,000 $CHCl_3$ solution plus 0.3 ng internal standard TCB plus 5 ng toxaphene
(c) New Orleans Drinking Water Extract. 2 µl of 1:25,000 $CHCl_3$ solution plus 0.3 ng internal standard TCB plus 15 ng toxaphene

15 ng toxaphene level, the toxaphene is apparent in both the 307 m/e and 343 m/e scans (compare with Figure 4(a)).

This extract sample was not cleaned up. If regular pesticide clean-up procedures were applied to the extract, even lower limits of detection probably could be achieved.

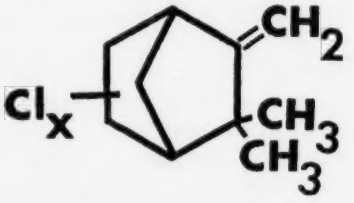
INTERFERENCES--DDE, TDE, DDT, AND PCB'S

DDE, TDE, and DDT are common interferences in the GC analysis for toxaphene. Figure 7(a) shows the GC-CI-MS output for a mixture of toxaphene, DDE, TDE, and DDT at relative concentrations of 10:1:1:1. The toxaphene is hardly recognizable. A gas chromatogram using electron capture as a detector would be similar. Using the SIM program, the DDT family would not interfere with toxaphene analysis since m/e 307 and m/e 343 are not in the CI spectrum of the DDT's. Figure 7(b) shows that an LMRGC of 307 m/e excludes DDE, TDE, and DDT (compare with Figure 7(c), the LMRGC of a pure toxaphene standard at 307 m/e).

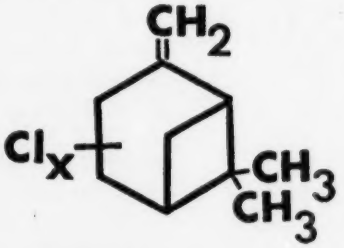
Similarly with the SIM program Arochlor 1260 does not interfere. Figure 8 shows the SIM program output for a mixture of 5 ng toxaphene and 5 ng Arochlor 1260. The presence of the Arochlor 1260 changes the baseline slightly (compare with Figure 4(b)), but the shape of toxaphene is still recognizable. Any of the lower Arochlors (1254, 1248, etc.) would interfere with quantitation since they contain the internal standard TCB.

DISTINGUISHING TOXAPHENE FROM STROBANE

The pesticide strobane has long been difficult to differentiate from toxaphene, although it is not as widely used as toxaphene. Toxaphene is manufactured by chlorination of camphene to a chlorine content of 67-69%. Strobane is manufactured by chlorination of a mixture of terpenes (mostly pinene) to a content of about 66% chlorine.



TOXAPHENE



STROBANE

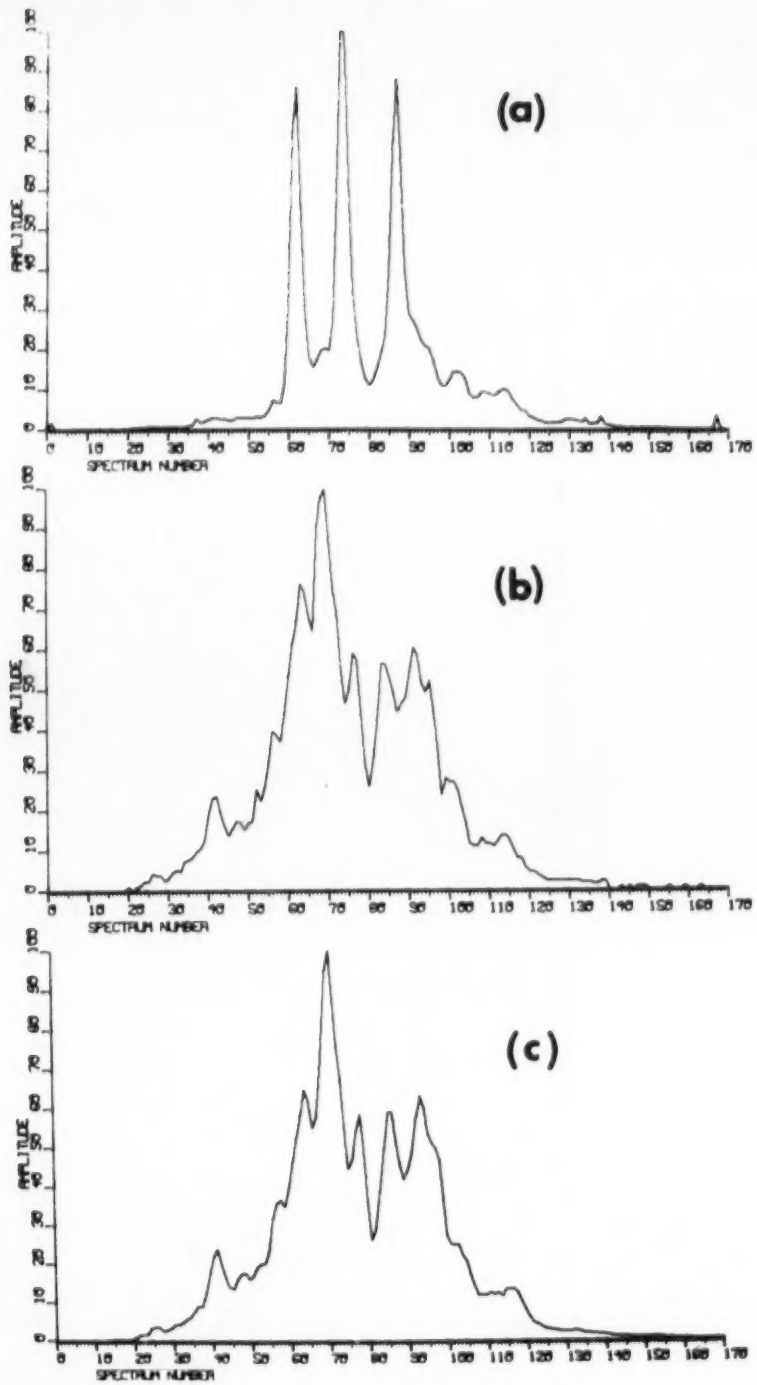


Figure 7. (a) Toxaphene + DDT's. RGC
(b) Toxaphene + DDT's. LMRGC at 307 m/e
(c) Toxaphene Standard. LMRGC at 307 m/e

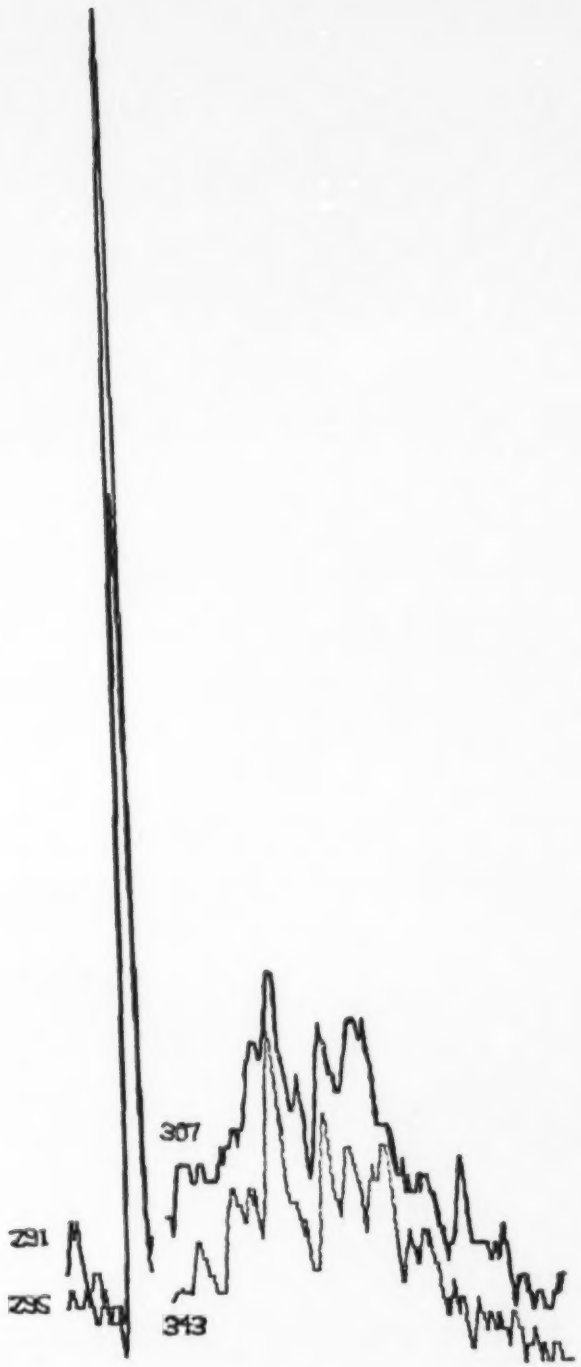


Figure 8. SIM Program Output, 0.3 ng Internal Standard TCB, 5 ng Toxaphene plus 5 ng Arochlor 1260

Both pesticides contain many isomers of the same molecular formula and therefore cannot be separated or distinguished easily from each other.

Chemical ionization LMRGC offers a means for distinguishing the two pesticides. Figure 9 shows the LMRGC's of toxaphene and strobane at 6 specific m/e's, superimposed on the RGC's of both. This gives a characteristic pattern for identifying or distinguishing the two pesticides.

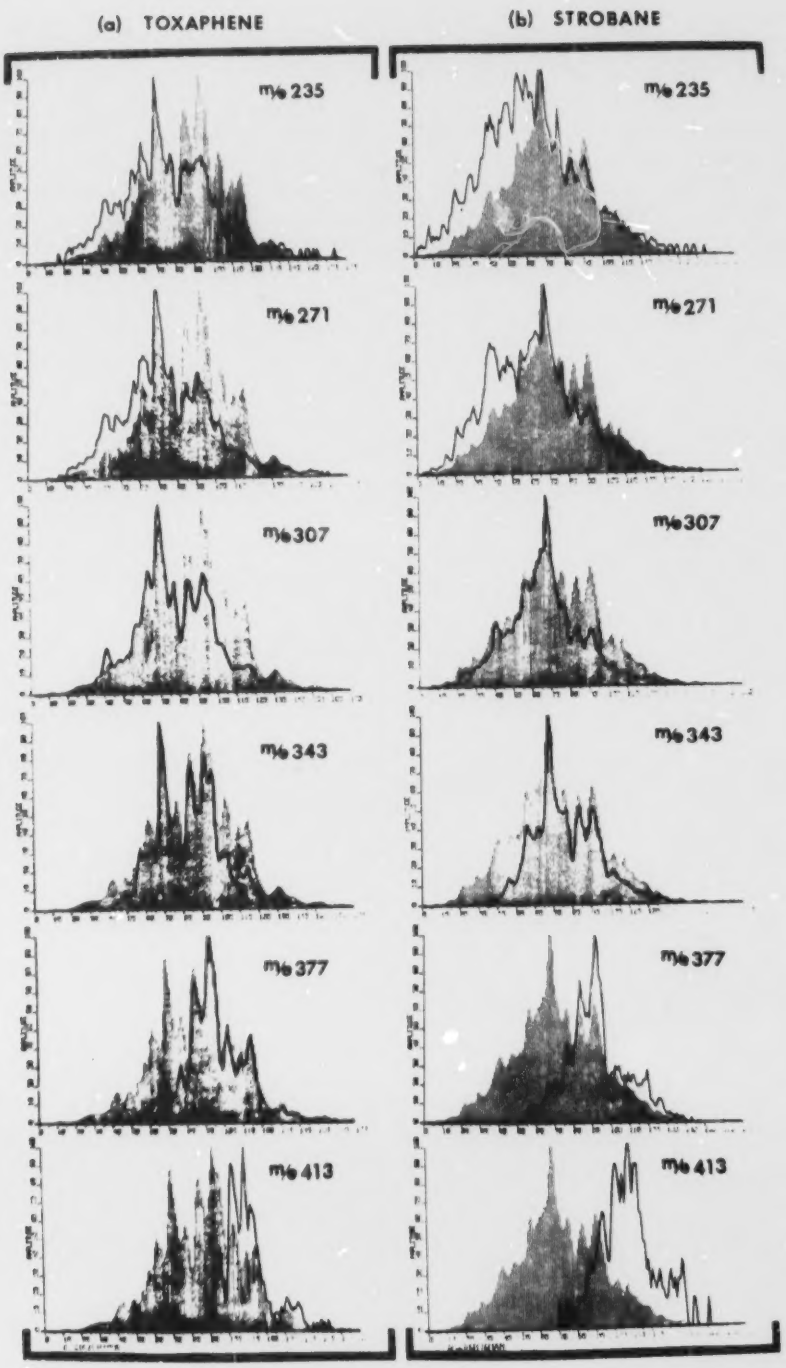


Figure 9. (a) Toxaphene LMRGC at 235,271,307,343,377, and 413 m/e. Shaded area is RGC
(b) Strobane LMRGC at 235,271,307,343,377, and 413 m/e. Shaded area is RGC

SECTION V

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SECTION VI

APPENDIX

PROCEDURE

The data aquisition is set up. The sample (2 µl) is injected into the GC operating isothermally at 160°C. After the solvent elutes (about 1 minute), the data program is initiated and the GC temperature program of 10°/min is initiated. The real time plot (Figure 10) appears on the plotter; the program monitors masses 291 and 295 of TCB, with only mass 291 showing on real time plot. Immediately after the TCB elutes, S2 is initiated manually by teletype and the next set of masses for toxaphene is monitored until the end of the run (only mass 307 showing on a real time plot). After the data acquisition is halted, data for all masses are plotted. These plots are normalized to the most intense signal (the internal standard). A standard curve should be set up keeping the internal standard the same, and varying the toxaphene. Quantitative values are plotted using peak heights by averaging the two highest peaks in the toxaphene plot.

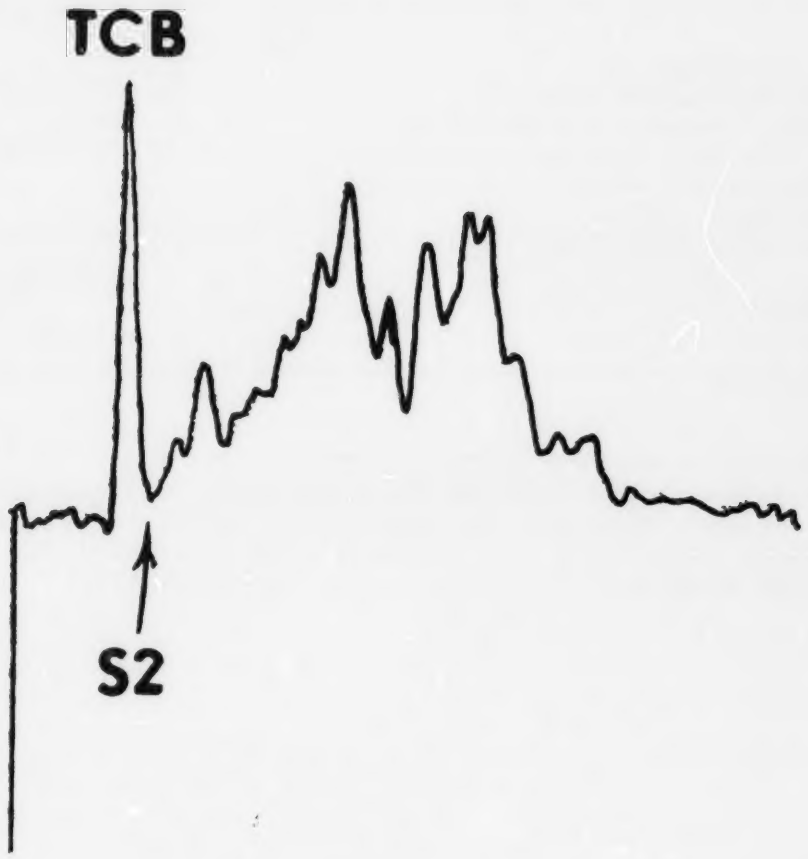

Figure 10. Real time plot of 0.3 ng TCB plus 15 ng toxaphene. S2 is initialed manually immediately after the TCB peak